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Likelihood Ratios to Apply for Nasal Bone, Ductus Venosus and Tricuspid Flow at the 11–13 Weeks' Scan in Down Syndrome Screening

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Key Words
Antenatal screening · Down syndrome · Ductus venous · Fetal nuchal translucency · First trimester · Noninvasive prenatal diagnosis · Ultrasound screening · Ultrasound markers · Nasal bone · Tricuspid flow

Abstract
Objective: To assess the feasibility of nasal bone (NB), ductus venosus (DV) and tricuspid flow (TF) at the 11–13 weeks' scan, calculate likelihood ratios for each of the markers and evaluate their efficacy in expanded and contingent screening strategies for Down syndrome. ***Material and Methods:*** NB, DV and TF were assessed in 11,261 singleton fetuses undergoing first trimester combined screening. For each marker, Down syndrome detection rate (DR), false positive rate (FPR), positive, negative and isolated likelihood ratios (PLR, NLR and iLR) were calculated. Likelihood ratios were multiplied to the combined test risk either to the entire population or to the intermediate risk group (expanded and sequential strategies, respectively). ***Results:*** Down syndrome was diagnosed in 101 pregnancies. Feasibility for marker assessment ranged from 71 to 97%, DRs for isolated markers from 20 to 54% and FPRs from 1.3 to 5.3%. PLR ranged from 10 to 15, NLR from 0.5 to 0.8 and iLR from 3.9 to 5.6. When ultrasound markers were added to both strategies, a significant FPR reduction was observed. ***Conclusion:*** The application of NB, DV and TF likelihood ratios to the combined test risk, either in an expanded or contingent strategy, result in a FPR reduction.

Introduction

In the last decade, the first trimester combined test, based on maternal serum Pregnancy Associated Plasma Protein A (PAPP-A), free-β human chorionic gonadotrophin (fβ-hCG) and fetal nuchal translucency (NT), has become the standard of care in Down syndrome screening in many countries and regions. For a 3–5% false positive rate, the combined test can identify up to 90% of all fetuses with trisomy 21 [1–3]. Adding first trimester ultrasound markers other than NT, such as the nasal bone (NB), ductus venosus (DV) and tricuspid flow (TF), has been demonstrated to improve the screening performance of the combined test [4–10]. They can be concurrently assessed in all pregnancies (expanded combined test), or sequentially in selected pregnancies, with either lower (stepwise sequential screening) or intermediate risk (contingent screening) [11–13]. Typically these additional markers may decrease the false positive rate resulting from the combined test [14, 15].

The purpose of the present study was to evaluate the feasibility and efficacy of the NB, DV and TF in Down

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syndrome screening at 11–13 weeks of gestation in our clinical setting and to provide the likelihood ratios derived from our data. Different combinations of these ultrasound markers were explored in the entire population (expanded combined test) or in the intermediate risk group (contingent screening).

Material and Methods

During a 8-year period (2002–2009), fetal NB, DV and TF were prospectively assessed in 17,141 singleton pregnancies undergoing first trimester combined screening for trisomy 21 at the Hospital Clinic of Barcelona (IRB: CEIC 02/1081). According to the aneuploidy risk level a the time of the scan, 86% percent of the population (n = 9,685) had no known risk factor. In this unselected population, the first trimester combined test was offered in a two-step approach (blood extraction at 8–10 weeks and NT measured at 11–13 weeks [3]). 10% of the population included (n = 1,146) were referred to our center (selected), mainly for an invasive procedure, due to identified aneuploidy risk factors, namely an estimated risk above 1/250 at the first combined test, advanced maternal age above 38 years (until 2008), abnormal ultrasound scan, or an NT above the 99th percentile. In the remaining 4% of the population (n = 430), no information about previous risk factors was available. In the whole population, a chorionic villus sampling was offered when one of the above factors were detected.

At 11–13 weeks, NB, DV and TF were assessed by appropriately trained sonologists by using three ultrasound machines (Power Vision 6000 SSA-370, Toshiba Medical Systems; Acuson Antares, Siemens Medical Solutions, Malvern, Pa., USA; and Voluson 730 Pro, GE Healthcare, Milwaukee, Wisc., USA). Ultrasound anatomic evaluation was carried out transvaginally (3.5 MHz) and resumed transabdominally if appropriate views for aneuploidy markers could not be obtained. The gestational age was derived from the fetal crown-rump length (CRL) [16]. NT and the other ultrasound markers (NB, DV and TF) were assessed in a maximum of 25 min scanning time following the Fetal Medicine Foundation guidelines (http://fetalmedicine.com/f-downs.htm). Results of NB, DV and TF were not used clinically, with the exception of fetal extended echocardiography being offered when absent or reversed A-wave was observed in the DV. NB was considered to be present when the lower line of the equal sign was more echogenic than the overlying skin, and absent if it was similarly or less echogenic than the skin line above, or not visible at all [4, 8]. NB hypoplasia was defined as NB length below 1 mm [17]. A 1.42 DV Pulsatility Index (DV PI) was adopted as the cut-off for abnormal flow, since this was the 95th centile in our series [6, 18]. Tricuspid regurgitation was diagnosed if it was lasting at least half of cardiac systole and velocities were above 60 cm/s [19].

Data on perinatal outcome was sought for the non karyotyped pregnancies. If the pregnancy was not delivered in our center, details of the pregnancy outcome were obtained by telephone either from the mother or the attending obstetrician. Pregnancies with: (a) neither perinatal outcome nor karyotype; (b) no combined test results; (c) other chromosomal anomalies than T21; and (d) multiple pregnancies were excluded from the study.

Statistical Analysis

Detection Rate (DR) and False Positive Rate (FPR) for Down syndrome were calculated for the 3 studied ultrasound markers, considered as dichotomous variables, as previously defined. The positive likelihood ratio (PLR) and negative likelihood ratios (NLR) were calculated as the DR/FPR and FPR/DR ratios, respectively. The isolated likelihood ratio (iLR) resulted from multiplying the PLR of the corresponding marker by the NLR of the remaining two markers. Subsequently, two different screening strategies were assayed with the use of these likelihood ratios. First, an expanded combined test was explored being Down syndrome risks recalculated for all study population, multiplying the combined test risk by the corresponding PLR or NLR for each of the 3 markers. Secondly, in the contingent screening, a similar modification of the previous trisomy 21 risks was carried out, but only in risks ranging from 1/101 to 1/1,000. Finally, both strategies (expanded combined and contingent) were explored with the use of different combinations of ultrasound markers as follows: individual markers (NB, DV, and TF), combination of 2 markers (NB + DV; NB + TF; DV + TF) and all 3 markers together. SPSS 19.0 (SPSS Inc., Chicago, Ill., USA) was used to calculate the 95% confidence intervals.

Results

Mean maternal age was 32.3 years (SD 5.4), 55% were primiparous, 17% of women were smokers, and the mean body mass index was 23.6 (SD 3.7). Ethnicity distribution was as follows: Caucasian (81%), South-American (10%), Pakistanese (4.4%), North-African (3.2%), Black-African (1.2%) and Asian (0.2%). The mean gestational age at the time of the scan was 12 + 0 weeks (11–13 + 6), being 62.5 mm (SD 10.13) the mean CRL, and 1.62 mm (SD 0.8) the mean NT. Among the 17,141 singleton pregnancies with an 11–13 weeks' scan, 16,284 had a complete follow-up. 462 pregnancies were excluded from further analysis due to chromosomal abnormalities other than T21, as well as 4,561 pregnancies with no biochemical data. The final sample included 11,261 singleton pregnancies with an ultrasound, biochemical and follow-up data. NB and DV assessment was assessed in the entire study period, while TF was assayed only during the last year of the study, accounting for the 1,113 fetuses (10%). In 1,453 pregnancies (13%) the combined test resulted in an intermediate risk (1/101–1/1,000). An invasive procedure was performed in 1,881 pregnancies (17% of the population), mainly by means of chorionic villus sampling (98.6%) and cytogenetic studies revealed 101 trisomies 21 (0.9%).

Regarding the feasibility of marker assessment, NB could be examined in 76% of pregnancies, DV in 97% and TF in 71%, as compared to NT which was obtained in 99.7% of our sample. In the 0.3% remaining pregnancies, the combined risk was estimated in the referring center.

Table 1. Detection and False Positive Rates (with 95% confidence intervals), and Positive and Negative Likelihood Ratios obtained by NB, DV and TF together with Nuchal Translucency. Isolated Likelihood Ratios are calculated with the Positive Likelihood Ratios of the marker and the Negative Likelihood Ratios of the remaining two markers

	Detection rate	False positive rate	PLR	NLR	iLR
Nuchal translucency					
Rate	69% (68/99)	5.0% (549/11,014)	–	–	–
95% CI	60–78	4.6–5.4			
Nasal bone					
Rate	20% (15/77)	1.3% (108/8,506)	15	0.82	3.9
95% CI	11–28	10.5–14.9			
Ductus venosus					
Rate	54% (50/93)	5.3% (572/10,830)	10.2	0.49	4.4
95% CI	44–64	4.9–5.7			
Tricuspid flow					
Rate	49% (17/35)	3.4% (37/1,078)	14.3	0.53	5.8
95% CI	32–65	2.4–4.5			

PLR = Positive likelihood ratio; NLR = negative likelihood ratios; iLR = isolated likelihood ratio.

The trisomy 21 DR achieved by each ultrasound markers were: 20% for NB, 54% for DV, and 49% for TF, all below the 69% NT DR observed in our series. The corresponding FPR for each marker were 1.3, 5.8 and 3.4%. PLR ranged from 10 to 15, whereas NLR from 0.5 to 0.8. The resulting iLR were 3.9, 4.4 and 5.8, respectively (table 1).

The combined test selected 92% of the Down syndrome fetuses, and 6.9% of the euploid pregnancies. The addition of the studied ultrasound markers to the combined test in the entire study population (expanded combined screening) and in the intermediate risk group (contingent strategy) resulted in a significant FPR decrease, maintaining a similar DR (table 2). Among the different combinations of added ultrasound markers, the inclusion of DV and NB + DV into the expanded combined screening and all combinations of ultrasound markers in the contingent strategy but NB alone, were found to be the most useful combinations to significantly reduce the FPR (table 2).

4 Discussion

To the best of our knowledge, this is the largest first trimester series to provide likelihood ratios for the 3 most commonly used ultrasound markers other than NT (NB, DV and TF). We aimed to apply these likelihood ratios when pregnant women request further information after the first trimester combined test (namely advanced maternal age, assisted reproduction, borderline risk) to refine risk estimation and no appropriate software is available. In those pregnancies, we suggest using the same methodology that Nicolaides [20, 21] proposed for second trimester ultrasound markers, applying positive and negative likelihood ratios for each of the markers.

Individual Performance and the Likelihood ratios of each Marker

In our series, we obtained DR for the 3 studied markers in the lower range of previously described results. Hence, DR for Doppler markers were lower (54% DV and 49% TR) than previously described (65–70%) [6, 7, 10], while FPR were similar (5.3% DV and 3.4% TR) [9, 19]. The marker with the lowest DR was observed for NB (20 vs. 50–60% reported in literature) [4, 22], although the FPR was also lower (1.3 vs. 2.5% described by Kagan and co-workers [8]). Low DRs may be explained by the fact that 18 different sonologists (the entire Ultrasound Unit) participated to the present study, in contrast to our previous studies conducted in a highly experienced unit (Prenatal Diagnosis Unit). Focusing on NB remarkably low DR, three facts may explain why in our center and in other reported studies [24], DR is well below 50%. Firstly, it is highly dependent on fetal position. In our center, we are unable to reschedule pregnancy scans exclusively for NB assessment. This may be a crucial factor in the comparison with other centers with better rates. Secondly, there is a known overlap between those fetuses where the NB 'cannot be examined' (due to the presence of the midline gap [23], inappropriate insonation angle or suboptimal ultrasound visualization) and those that are 'absent'. A third specific limitation for NB assessment is its ethnical variability [22].

One of the main contributions of our study is the calculation of likelihood ratios for individual first trimester ultrasound markers by using the established method for second trimester ultrasound markers, without requiring specific software [20, 21]. In this regard, Down syndrome risk decreases between 20 to 50% (NLR ranging from 0.49 to 0.82) with normal markers, while in abnormal markers this risk increases about 10 to 15 times (PLR ranging from 10.2 to 15.0). When a single marker is abnormal and the remaining 2 are normal, the resulting risk may increase up to 6-fold.

Expanded Combined Test and Contingent Strategy

In our study, the most remarkable advantage of adding ultrasound markers to the combined test, either to the entire or only to intermediate risk population, is a 29 or 33% FPR reduction. Regarding the expanded combined test, an improved screening efficacy has been reported in previous studies, obtaining both an increased DR and a reduced FPR (10–29%) [15, 25]. In our series, only the addition of DV or NB ± DV to the combined test resulted in a reduction of FPR by about one fourth (25–29%), similar to that reported in a previous study [14]. Concerning the contingent screening approach, in our series the use of DV ± TF resulted in a significant 42% FPR reduction (from 6.9 to 4.0%) with a marginal DR increase. Similarly, in most of the reported series, greater FPR reductions were achieved in the contingent approach as compared to the expanded combined [5, 11, 12], although the DR may decrease [12]. The main advantage of the contingent approach is that additionally marker assessment is required only in about 10% of pregnancies, being considered the most cost-effective screening strategy in most of the studies [26]. A serious drawback of contingent screening was recently demonstrated by our group, when largely applied in public Catalan health service, because half of the pregnancies with low intermediate risks could not be offered the second stage before 14 weeks due to time constraints [11].

Weakness and Strengths of the Study

Weaknesses of our study limiting the applicability of our results to other centers are related to the characteristics of the study population, since 10% of our study population was at high risk for fetal aneuploidy. However when LRs were recalculated in the subgroup of unselected pregnancies (49 trisomies 21 among 9,685 pregnancies), no major differences were observed (data not shown). A second limitation of our study is the low success rates for NB (76%) and TF (71%) assessment, supporting that each center should decide either in which marker to be included and the strategy elected according to their own experience, economic budget and cost-effectiveness of the strategy. A third limitation is related to the method for LR computation not taking into account changes with gestational age and the interrelation between markers. This can be solved with the use of a proper software, but the aim of our study was to apply to first trimester ultrasound markers the same simplified method proposed by Nicolaides [20, 21] for second trimester ultrasound markers.

The main strength of our study is the high external reproducibility. as all the routine scans were performed by

Table 2. Detection and False Positive Rates (with 95% confidence intervals) achieved with the addition of NB, DV and TF to the combined test risk either in the entire study population (expanded combined test) or in the intermediate risk group (contingent strategy)

		Detection rate	False positive rate
None (combined test)	Rate 95% CI	92% (93/101) 87–97	6.9% (769/11,160) 6.4–7.4
Expanded combined test	Rate 95% CI	89% (87/98) 83–95	4.9% (542/11,050) 4.5–5.3*
Contingent screening	Rate 95% CI	91% (92/101) 86–97	4.6% (551/11,142) 4.5–5.4*
Different marker combinations for an expanded combined test			
NB	Rate 95% CI	92% (71/77) 86–98	6.5% (557/8,506) 6.0–7.1
DV	Rate 95% CI	92% (86/93) 87–98	5.2% (565/10,830) 4.8–5.6*
TF	Rate 95% CI	94% (33/35) 87–100	13% (140/1,078) 11.0–15.0
NB + DV	Rate 95% CI	93% (67/72) 87–99	4.9% (406/8,289) 4.4–5.4*
NB + TF	Rate 95% CI	96% (27/28) 90–100	12% (93/779) 9.7–14.2
DV + TF	Rate 95% CI	88% (30/34) 77–99	8.4% (88/1,053) 6.7–10.0
NB + DV + TF	Rate 95% CI	89% (24/27) 77–100	9.9% (75/760) 7.8–12.0
Different marker combinations for a contingent screening			
NB	Rate 95% CI	93% (92/99) 88–98	5.6% (604/10,766) 5.2–6.0*
DV	Rate 95% CI	93% (93/100) 88–98	4.7% (526/11,099) 4.3–5.1*
TF	Rate 95% CI	96% (90/94) 92–100	4.3% (423/9,924) 3.9–4.7*
NB + DV	Rate 95% CI	94% (92/98) 89–99	4.2% (452/10,726) 3.8–4.6*
NB + TF	Rate 95% CI	97% (90/93) 93–100	3.9% (388/9,881) 3.5–4.3*
DV + TF	Rate 95% CI	96% (90/94) 92–100	4.0% (399/9,914) 3.6–4.4*
NB + DV + TF	Rate 95% CI	97% (90/93) 93–100	4.0% (396/9,875) 3.6–4.4*

* Not overlapping confidence intervals when compared with the Combined test.

NB = Nasal bone; DV = ductus venosus; TF = tricuspid flow.